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Dear Mike,

Another progress report! Marianne has, I believe, already let you know that the cells arrived in good shape and she will work them up soon to isolate the DNA's for testing. Thanks for making that part of the experiment easier.

Meanwhile we have run through a few more clones and repeated the annealing kinetics with the older ones. The results are quite consistent and give the same result (Table I); none of the abortively transformed BHK have any trace of PY DNA! In Cot #17 the $Cot_{1/2}$ would have decreased by a 2-fold if the cell DNA had contained 2.5ng of viral DNA; assuming the diploid cell genome size is $\sim 3 \times 10^{12}$ daltons of DNA, halving of the $Cot_{1/2}$ would have indicated 1 viral genome/cell. In Cot #20 we increased the sensitivity so that 1.2ng of viral DNA in 2.5mg of cell DNA would have halved the Cot $1/2$. As you can see except for clone MT-1 there is no substantial homologous sequences in any of these abortives. ST-1 (our batch of cells) is puzzling; one could argue that it has about ≤ 0.5 a viral equivalents but I don't know whether to believe it. We are repeating it with DNA isolated from the ST-1 you just sent us (and which you said is T-ag negative although morphologically transformed). We plan to run through the rest of the abortives and stables we have to get more numbers but then there is the question of interpretation and what next!

If the result is real (and I tend to believe it at this point) and Smith and Martin's result for SV40 abortively infected cells is also correct, then what is it trying to tell us? Is it that the steps and mechanism of transformation by the two viruses in these hosts is different? Conceivably in PY the integration-excision function (ts-a?) is expressed all the time, both in the "free" and "integrated" state so that a DNA molecule is constantly integrating and excising. Stable transformation occurs only when an integrated genome can't be excised (even though excision "enzyme" is present) or when a genome with a defective ts-a function is integrated and there is a lack of helper particles to complement its excision. In short, wild-type particles can't remain integrated (unless aberrent insertion) but DNA's with a defective ts-a function (as for examples Mike Fried's ts-a itself at high temperature) remain integrated. This model is quite consistent with Vogt and Summers' and Bill Folk's

recent findings on the "inducibility" of ts-a transformed BHK. In one sense BHK may not be any different than mouse cells except for the inability to multiply the virus. That is, PY BHK is like PY-3T3 and ts-a-BHK is like ts-a-3T3 except that 3T3 is permissive for extensive replication.

What about SV40 infection of 3T3? Perhaps in this instance the integration-excision function is inactivated as a result of integration; that is, integration shuts off expression of the gene controlling the excision function. Thus when growth of the cell dilutes out existing enzyme and helper particles, the integrated genome becomes "locked in". Fusion with permissive cells could activate the excision function and permit replication to occur.

It's quite possible that there are clones of abortively transformed BHK that do contain PY DNA but these might be rare and represent only that class in which either the excision function was defective or the mode of integration was abnormal; in either case the transformed phenotype would have to be "repressed".

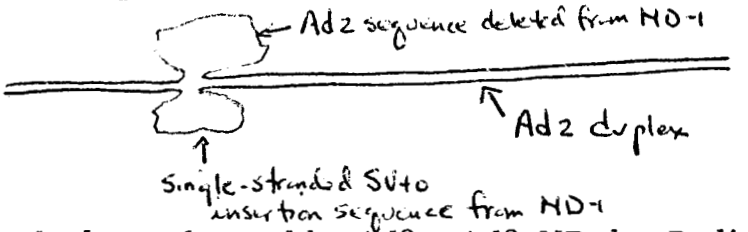
The only way I can think of to test this model is to look for curing of transformed cells. I used to think it was best to do this by superinfecting PY-BHK with PY (at high moi) and to see if substantial numbers of non-transformed clones are produced (infection should catalyse a new round of excision-integration leading to a new stable state, about 5% transformed cells). We took a try at this but the attempt was crude, being done with only one strain of PY-BHK; we have not done more. But now I think it should be tried with SV3T3 superinfected with SV40 at multiplicities which cause good "abortive transformation" I shall approach Helene Smith about doing that experiment.

What do you think? and what do you suggest we do further with the analysis of the PY-BHK abortives? Is it worthwhile making another batch of abortives to test? That would take time but maybe it should be done to nail the point down. Perhaps one should use mutagenized PY or preps enriched for defective virus. Conceivably one could reduce the excision process and thereby hope to produce abortives that contain viral DNA.

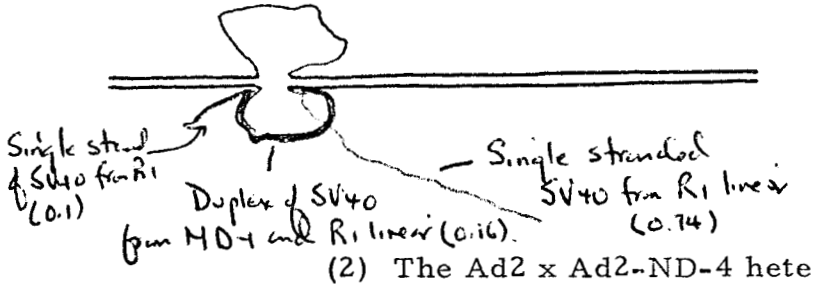
Are you going to be in the States this summer? At the Tumor Virus meetings in Cold Spring Harbor or at a Gorden Conference? Or is the only way we can discuss this or plan something for me to come to London for awhile? I turned down going to the Brighton Cell Biology meeting (thanks for suggesting my name to them as a speaker) because the first two weeks of September was a very bad time for me to be away. If you're not going to be in the U.S. do you believe it would be worthwhile for me to come during the second half of September for long enough to review and discuss the experiments and how to proceed from here. (Unfortunately I don't think I can stay long enough to do any serious experiments.)

Now for other news. You may recall that I wrote you about the R_I restriction enzyme making one specific double-strand scission of SV40 to create a unique length linear molecule. We've used that molecule now to locate the SV40 segments carried by Ad2-SV40 hybrid ND-1, ND-4 and E46$^+$ along the R_I linear.

(1) For example the Ad2 x Ad2-ND-1 heteroduplex looks as follows:

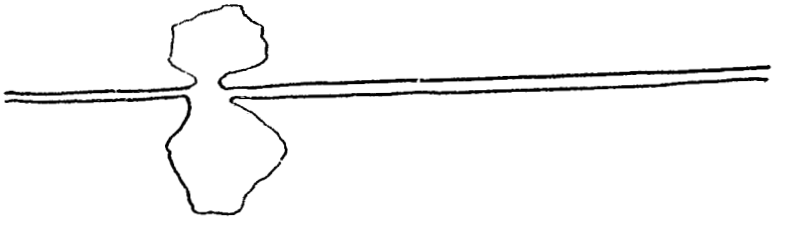


and the heteroduplexes formed by Ad2 x Ad2-ND-1 x R_I linears of SV40 looks like

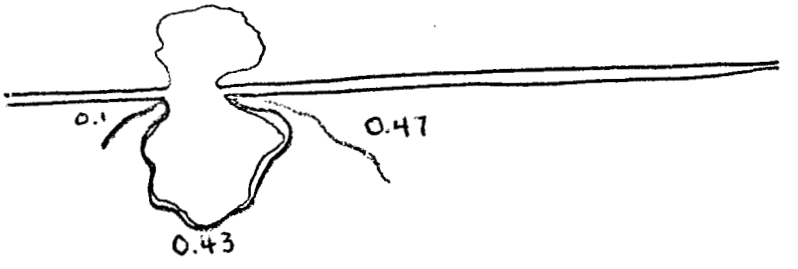


the short arm (0.1) plus the short duplex region (0.16) and the long arm (0.74) equal one SV40 length.

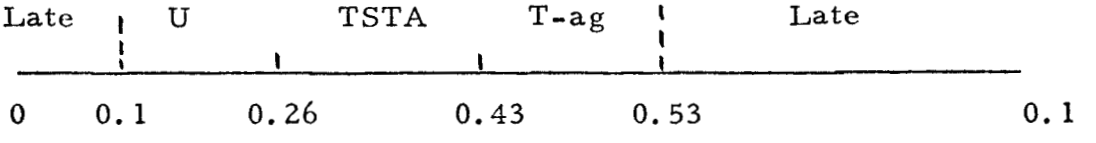
(2) The Ad2 x Ad2-ND-4 heteroduplex is as follows:



and the corresponding Ad2 x Ad2-ND-4 x R_I linear is

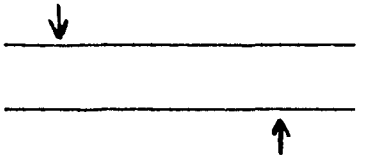


According to Lewis and Kelly the AD2-ND-1 induces U-ag while Ad2-ND-4 induces U, TSTA and T-ag. Moreover they say that little or none of the late SV40 genes are carried in these hybrids. Therefore it follows that the R_I restriction enzyme cleaves in one of the "late" genes of SV40 perhaps one of the capsid structural genes. One could draw a tentative "genetic map" of SV40 (not to be taken too seriously) as follows. The actual boundaries of

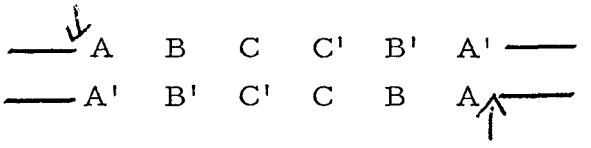


precise boundaries of the
The three putative genes are unclear although the order seems to derive from the fact that ND-1 induces U alone, ND-2 induces U and TSTA but not T and ND-4 induces all three antigens. It is interesting that the segment assigned to late genes is enough to code for about 70,000 daltons worth of polypeptide which would account for the two chains 40,000 and 30,000 that Renato says comprise the capsid.

The heteroduplexes with E46$^+$ are more complex but consistent with this model. The difference is that the SV40 segment integrated in the E46$^+$ hybrid has a deletion of the R_I restriction site but the early genes appear to be contiguous.

The most recent finding which excites us is that R_I makes a staggered break, i.e.,  in which the number of bases between

breaks is of the order of 6 ± 2 (probably six). Thus the linears can be circularized at low concentration at 3-5°C and can be covalently sealed with DNA ligase to regenerate completely infectious and full length SV40 molecules (actually the linears are one-tenth as infectious as wild-type probably because the cell itself can't "close the ring"). It seems very likely that the site at which the enzyme cleaves is identical in all DNA's (it occurs on the average once per 4,000 bases in a random sequence) and very likely is symmetrical.



We can show experimentally, that any two DNA molecules having ends produced by R_I endonuclease can be covalently joined. In other words the ability to construct molecular hybrids is enormously extended. We have now to find out how to deal with this intelligently.

How is the work going on the serum factors? Is it possible to assign specific cellular functions to specific serum proteins yet? You must be just about getting ready to move to the new quarters. Good Luck. I envy Art Pardee his next year.

Well so much for now. I look forward to hearing from you about PY abortives. As we get more data I'll keep you in touch. I'd appreciate it if you could pass some of this scientific information along to Bill Folk and anybody else you care to so as to save me the time of writing it to him.

With best regards to all at ICRF and to Veronica.

Sincerely yours,

Paul

(see next page)

PB:af

P.S. I wonder if I can make a request of you. So far we've been using one of our PY virus stocks to prepare the P^{32} DNA for annealing. That's not the same one we used to do the infections of BHK. I don't believe that's serious but perhaps we should make some DNA from your stock to be sure. More importantly our virus stock must surely contain defective particles containing insertions of host DNA. This slightly complicates the kinetics of annealing (because the host sequences probably don't anneal with the same kinetics as the PY sequences). If your stock is plaque purified or if you have a plaque purified stock from which you could let us have enough to make a batch of DNA (from infected mouse kidney cells) that would be very helpful. Otherwise we shall have to take the time to make such an isolate and that would be time consuming.

TABLE I

Cell DNA's tested[1]	Cot #17[2]	Cot #20[3]
	Normalized $Cot_{1/2}$	
Salmon Sperm	2.1×10^{-3}	1.3×10^{-3}
BHK	2.1×10^{-3}	1.3×10^{-3}
SA-2		1.9×10^{-3}
SA-10	2.0×10^{-3}	1.4×10^{-3}
MA-4		1.3×10^{-3}
MA-6		1.3×10^{-3}
MA-8	1.9×10^{-3}	1.4×10^{-3}
ST-1	1.6×10^{-3}	0.9×10^{-3}
MT-1	0.6×10^{-3}	

1. Cell DNA's were added to the annealing mixture at a concentration of $50A_{260}$ (2.5mg/ml); it was sheared to average single-strand chain length of 400-500 bases.

2. In Cot #17 the ^{32}P-PY DNA was at a concentration of $5 \times 10^{-5}A_{260}$ (2.5ng/ml); average chain length of DNA was 400-500 bases.

3. In Cot #20 the ^{32}P-PY DNA was at a concentration of $2.5 \times 10^{-5}A_{260}$ (1.2ng/ml); average chain length same as in Cot #17.